IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Chatterjee et al.

Serial No.: 08/372,676

Filing Date: 01/07/95

For: Anti-idiotype monoclonal antibody 1A7 and use for the treatment of melanoma and small cell carcinoma

Examiner: J. Reeves

Group Art Unit: 1813

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, SUNIL K. CHATTERJEE, Ph.D., do hereby declare as follows:

1. I have been a collaborating investigator with Malaya Chatterjee and Kenneth Foon, inventors for the above-referenced patent application.

2. I am a Member of the Markey Cancer Center in Lexington, and am an Associate Professor in the Department of Obstetrics and Gynecology, University of Kentucky. My research expertise includes the field of molecular biology and genetic engineering. A copy of my *curriculum vitae*, describing my background and qualifications, accompanies this Declaration as *Exhibit C.*

3. I have obtained the nucleic acid sequence and the corresponding amino acid sequence for the heavy and light chain variable regions of monoclonal antibody 1A7. This data, along with the method used to obtain it is provided in *Exhibit A* attached to this declaration.

4. The heavy and light chain amino acid sequences were compared using the BLAST algorithm at the National Center for Biotechnology Information with all sequences available from the PDB, SwissProt, PIR, SPUpdate, GenPept, and GPUpdate databases. The comparison was performed on December 16, 1995.

5. Amongst the 50 database sequences matched most closely to that of the 1A7 light chain variable region, none was identical. 1A7 differed from the five closest sequences by **2** substitutions at residues 50 and 55, which are contained in the second complementarity determining region (CDR2). The two differences at these positions were non-conservative substitutions, and persisted in comparisons with other light chain sequences.

Panel A of *Exhibit B* provides a comparison of the 1A7 light chain sequence with the 15 closest sequences found in the BLAST search. Residues identical to those in 1A7 are indicated with a period.

6. Amongst the 50 database sequences matched most closely to that of the 1A7 heavy chain variable region, none was identical. The following summarizes the main points deduced from the comparison.

- The closest match was with a heavy chain fragment beginning at residue 9 (designation gp|M36221|MUSIGHAEB_1). There were **6** substitutions between

residues 1 and 97 (before the VDJ junction), **6** substitutions after residue 97, and 1A7 was shorter about the VDJ junction by **2** residues.

- The closest match with a full length heavy chain variable region had the following features (designation gp|U01185|MMU01185): There were **10** substitutions between residues 1 and 97, **7** substitutions after residue 97, and 1A7 was shorter about the VDJ junction by **3** residues.
- 1A7 differed in length from all sequences but one, due to insertions or deletions of 1 to 8 residues about the VDJ junction. For the sequence of equal length (designation pir|S11106|S11106), there were **18** substitutions between residues 1 and 97, and 8 substitutions after residue 97.
- All other comparisons showed at least **14** differences between residues 1 and 97.
- All other comparisons showed at least **4** differences after residue 97.
- All other comparisons showed a total of at least **22** substitutions, insertions or deletions along the entire variable region.
- Differences appeared throughout the variable region.

Panel B of *Exhibit B* provides a comparison of the 1A7 heavy chain sequence with the 15 closest sequences found in the BLAST search.

7. Amino acid consensus sequences of the 15 most closely matched V_L and V_H regions were designed, and compared with the 1A7 sequences. This is shown in Panel C of *Exhibit B.* Identical residues are marked with a period, and CDRs are overscored with asterisks.

Other than splicing differences about the VDJ junction, there appear to be about **16** differences between 1A7 and the prototype sequences. Two of these differences are present in

the light chain; **14** are present in the heavy chain. Seven differences occur in the CDRs, while nine occur in the variable region framework.

8. The sequence data described herein were obtained no earlier than about July 24, 1995. The 1A7 sequence data have not been disclosed except under terms of confidentiality. The data were included in a recent grant application made to the National Institutes of Health under terms of confidentiality, and it is my understanding that the data will remain confidential until the grant is approved. It is my understanding that a decision on the application has not yet been rendered.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

3/8/96
Date

Sunil Chatterjee
Sunil Chatterjee, Ph.D.

Exhibit A:

Sequences of 1A7

The polynucleotide sequences were obtained for the 1A7 antibody by isolating messenger RNA from the 1A7 producing cell line. For each sequence determination, total RNA was isolated from ~1 x 10^6 1A7 hybridoma cells. The yield of total RNA was about 100 μg. First strand cDNA was synthesized using SuperScript Preamplification kit (GIBCO/BRL).

To sequence the heavy chain variable region, PCRs were conducted on the cDNA using a reverse primer corresponding to amino acids 126 to 119 of the murine γ_1 constant region:

5'-CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG -3'

and various mixtures of forward primers, corresponding to the *N*-terminal leader sequences of murine variable region subgroups. The forward primer that gave a positive reaction was:

5'-ACTAGTCGACATGGCTGTCYTRGBGCTGYTCYTCTG-3'

corresponding to amino acids -20 to -12.

The amplified fragment of cDNA was subcloned into pT7 plasmid and NovaBlue competent cells were transformed using a protocol provided by the supplier (Novagen). Recombinant colonies were picked up by color selection and plasmid DNA

was prepared by miniprep procedure. The DNA sequence of the double stranded plasmid was determined using a Sequenase Version 2.0 kit (USB, Cleveland, Ohio). The sequence of the DNA insert in the plasmid was determined from both orientations using primers specific for the plasmid; namely T7 promoter (TAATACGACTCACTATAGGG) and U-19 (GTTTTCCCAGTCACGACGT). At least 8 clones were picked for sequence determination.

The sequence of the 1A7 light chain variable region was determined in a similar fashion. The forward and reverse primers giving a positive result in the PCR were:

5'-ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT-3'

5'-CCCAAGCTTACTGGATGGTGGGAAGATGGA-3'

corresponding to amino acids -19 to -10 of the leader sequence, and 122 to 116 of the mouse κ chain constant region.

The nucleic acid sequence and the corresponding translation for the light and heavy chain variable regions of monoclonal antibody 1A7 (along with neighboring residues of the leader and constant regions) are as follows:

1A7 light chain sequence

```
 M   K   L   P   V   R   L   L   V   L   M   F   W   I   P   A
ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GCT
 S   S   D
TCC AGC GAT  (-1 to -19, leader)

 D   V   L   M   T   Q   T   P   L   S   L   P   V   S   L   G
GAT GTT TTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA
 D   Q   A   S   I   S   C
GAT CAA GCC TCC ATC TCT TGC  (1-23, Frame work 1)

 R   S   S   Q   S   I   V   H   S   N   G   N   T   Y   L   E
AGA TCT AGT CAG AGC ATT GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA
(24-39, CDR 1)

 W   Y   L   Q   K   P   G   Q   S   P   N   L   L   I   Y
TGG TAC CTA CAG AAA CCA GGC CAG TCT CCA AAC CTC CTG ATC TAC
(40-54, Frame work 2)

 F   V   S   N   R   F   S
TTT GTT TCC AAC CGA TTT TCT (55-61, CDR 2)

 G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA
 L   K   I   S   R   V   E   A   E   D   L   G   V   Y   Y   C
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC
(62-93, Frame work 3)

 F   Q   G   S   H   V   P   W   T
TTT CAA GGT TCA CAT GTT CCG TGG ACG
(94-102, CDR 3)

 F   G   G   G   T   K   L   E   I   K
TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA
(103-112, Frame work 4)

 R   A   D   A   A   P   T   V   S   I   F   P   P
CGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA

 S   S   K   L   G
TCC AGT AAG CTT GGG  (Constant region)
```

1A7 heavy chain sequence

```
 M   A   V   L   G   L   L   F   C   L   V   T   F   P   S   C
ATG GCT GTC TTG GGG CTG CTC TTC TGC CTG GTG ACA TTC CCA AGC TGT
 V   L   S
GTC CTG TCC  (-1 to -19, Leader)

 Q   V   Q   V   K   E   S   G   P   F   L   V   P   P   S   Q
CAG GTG CAG GTG AAG GAG TCA GGA CCT TTC CTG GTG CCC CCC TCA CAG
 S   L   S   I   T   C   T   V   S   G   F   S   L   T
AGC CTG TCC ATC ACA TGC ACT GTC TCA GGG TTC TCA TTA ACC
 (1-30, Frame work 1)

 T   Y   G   V   S
ACC TAT GGT GTA AGC  (31-35, CDR 1)

 W   I   R   Q   P   P   G   K   G   L   E   W   L   G
TGG ATT CGC CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG GGA
(36-49, Frame work 2)

 A   I   W   G   D   G   T   T   N   Y   H   S   A   L   I   S
GCA ATT TGG GGT GAC GGG ACC ACA AAT TAT CAT TCA GCT CTC ATA TCC
(50-65, CDR 2)

 R   L   S   I   S   K   D   N   S   K   S   Q   V   F   L   K
AGA CTG AGC ATC AGC AAG GAT AAC TCC AAG AGC CAA GTT TTC TTA AAA
 L   N   S   L   Q   T   D   D   T   A   T   Y   Y   C   A   K
CTG AAC AGT CTG CAA ACT GAT GAC ACG GCC ACG TAC TAC TGT GCC AAA
(66-97, Frame work 3)

 L   G   N   Y   D   A   L   D   W
CTG GGT AAC TAC GAT GCT CTG GAC TAC
(98-106, CDR 3)

 W   G   Q   G   T   S   V   T   V   S   S
TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
( 107-117, Frame work 4)

 A   K   T   T   P   P   P   V   Y   P   L   V   P   G   S   L
GCC AAA ACG ACA CCC CCA CCC GTC TAT CCA TTG GTC CCT GGA AGC TTG GG
(Constant region)
```

Exhibit B

Comparison of 1A7 light chain variable region with database sequences

```
1A7:      1  DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPNLLIYFVSNRF  60

 1        1  ..........................................................K....K.....  60
 2        1  ..........................................................K....K.....  60
 3        1  ..V.......................................................K....K.....  60
 4        1  ..........................................................K....K.....  60
 5        1  ..........................................................K....K.....  60
 6        1  ..........................................................K....K.....  60
 7        1  ..........................................................K....K.....  60
 8        1  ..........................................................X..K....K.....  60
 9        5  ..............................S...F.....................K....K.....  64
10        1  ..........................................................K....K.....  60
11        1  ..........................................................K....K.....  60
12       20  ..........................................................K....K.....  79
13        1  ..........................................................K....K....L  60
14        1  ..........................................................K....K.....  60
15        5  ..............................S...F.....................K....K.....  64


1A7:     61  SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPWTFGGGTKLEIK  112

 1       61  ....................................................................  112
 2       61  ....................................................................  112
 3       61  ....................................................................  112
 4       61  ....................................................................  111
 5       61  ....X...............................................................  112
 6       61  ...........................................Y........................  112
 7       61  .........C..........................................................  111
 8       61  ....................................................................  111
 9       65  ...................................T................................  116
10       61  ......................R.....................Y........................  112
11       61  ...........................................R........................  112
12       80  ...........................................Y...S....................  131
13       61  ...........................................Y........................  112
14       61  .......T.............................W......Y........................  112
15       65  ............................Q........T................................  116
```

DATABASE REFERENCE:

1	gp| M34588	MU SIGKABR_1	Mouse Ig kappa-chain mRNA V-J regi...
2	gp| L18941	MU SIG438B_1	Mouse rearranged immunoglobulin li...
3	gp| Z22035	MD IGKVAH_1	immunoglobulin variable region [Mu...
4	gp| M32857	MU SIGKCSP_1	Mouse Ig rearranged kappa-chain mR...
5	gp| M34589	MU SIGKABS_1	Mouse Ig kappa-chain mRNA V-J regi...
6	gp| J04438	MUSIGKCWA_1	Mouse Ig-kappa chain (PAC1) mRNA V...
7	gp| M31271	MUSIGKCSM_1	IgM gene product [Mus musculus]
8	gp| M32858	MUSIGKCSQ_1	Mouse Ig rearranged kappa-chain mR...
9	gp| U29428	MMU29428_1	anti-PC Ig kappa chain [Mus musculus]
10	gp|X65770	MMIGMMM4_1	IgM gene product [Mus musculus]
11	gp|M83723	MUSIGKD2A_2	immunoglobulin kappa-chain VK-1 [M...
12	pir|B39276	B39276	Ig light chain precursor V-D-J reg...
13	gp|L14370	MUSIGKJVSA_1	immunoglobulin kappa chain [Mus mu...
14	pir|A31807	A31807	Ig kappa chain V region (PAC1) - m...
15	gp|U29267	MMU29267_1	IgL rearranged kappa chain V-J reg...

Comparison of 1A7 heavy chain variable region with database sequences

```
1A7:        1  QVQVKESGPFLVPPSQSLSITCTVSGFSLTTYGVSWIRQPPGKGLEWLGAIWGDGTTNYH   60

 1          1  .G..A.................S.....V.............V.....S....   52
 2          1  ...LQ....G..A.................S..IT.V.............V.....N....   60
 3         20  ...L.....G..A.................G...N.V.............T...N.S.D.N   79
 4          1  ...L..T..G..A.................S...H.V...........VV..S..S...N   60
 5          1  ...L.....G..A.................S...H.V.............V..AG.S...N   60
 6          1  ...L.....G..A.................S...H.V.............V..AG.S...N   60
 7          1  ...L.....G..A..............P..S...D.V.............V...G.S...N   60
 8         23  ...LQ....G..A.................G...N.V.............M.....N.D.N   82
 9          1  ...L.....G..A.................G...N.V.............M.....N.D.N   60
10        133  ...LQ....G..A.................G...N.V.............M.....N.D.N  192
11         20  ...L.....G..A.................G...N.V.............M.....N.D.N   79
12          1  ...L.....G..A................SR.S.H.V.............M...G.N.D.N   60
13         21  .HL.....V..A.................N...H.V.............V..AG.N...N   80
14         23  ...LQ....G..A.................G...N.V.............M.....N.D.N   82
15          1  ...LQ....G..A.................G...N.V.............M.....N.D.N   60


1A7:       61  SALISRLSISKDNSKSQVFLKLNSLQTDDTATYYCAKL--------GNYDALDWWGQGTSVTVSS  117

 1         53  ....................................P-----YDYExxxxx.Y.....TL..    109
 2         61  ....................................x-----xxxxxxx.K.Y.........    120
 3         80  .T.K...T.T...........M.........R....SVSIYYYGRSDK.FT..Y.........    144
 4         61  ...K.................M.........M....Rx------xx.D.Y.M.Y.........    119
 5         61  ...M.................M.........M....Rx----xxxxxx.Y.M.Y.........    120
 6         61  ...M.................M.........M....Rx------xxxx.Y.M.Y.........    118
 7         61  ...M.................M...X.....M....xx-----xxx.X.Y.M.Y.........    119
 8         83  ...K.................M...H.....R....RE--------=RDYR..Y.....T.....    138
 9         61  ...K.................M...H.....R....RE--------=RDYR..Y.....TL....    116
10        193  ...K.................M...H.....R....RE--------=RDYR..Y.....T.....    248
11         80  ...K.................M...H.....R....RE--------=RDYR..Y.....TL....    135
12         61  ...K.................M.........M....RD-------GYYDx.M.Y.........    117
13         81  ...M.................M....I....I....=x-----xxxxx.Y.M.Y.........    139
14         83  ...K.................M...H.....R....RE--------=RDYR..Y.....T.....    138
15         61  ...K.................M...H.....R....RE--------=RDYR..Y.....T.....    116
```

DATABASE REFERENCE:

```
 1   gp|M36221|MUSIGHAEB_1   immunoglobulin heavy chain V-region
 2   gp|U01185|MMU01185_1    immunoglobulin heavy chain [Mus mu...
 3   sp|P01819|HV43_MOUSE    IG HEAVY CHAIN PRECURSOR V REGION ...
 4   gp|M26985|MUSIGH1PR_2   Igh gene product [Mus musculus]
 5   gp|M36217|MUSIGHADX_1   immunoglobulin heavy chain V-regio...
 6   gp|M36228|MUSIGHAEI_1   immunoglobulin heavy chain V-regio...
 7   gp|M34626|MUSIGHACK_1   Mouse Ig rearranged heavy chain (N...
 8   gp|A05515|A05515_1      Vector pSW2HPOLY DNA sequence. [un...
 9   pdb|1FDL|H              IgG1 Fab Fragment (Anti-Lysozyme A...
10   gp|L43544|MUSALCA_1     antibody [Mus musculus]
11   gp|A03907|A03907_1      antibody D1.3 V region (VDJ) [Homo...
12   pir|S38563|S38563       Ig heavy chain V region (ASWS1) - ...
13   pir|A32456|A32456       Ig heavy chain precursor V region ...
14   gp|A05504|A05504_1      pSW1 protein [unidentified] >gp|A0...
15   gp|L43544|MUSALCA_3     Mus musculus (clone pCT.kvhd1) ant...
```

Consensus analysis

```
                                        ****************                  ******
VL consensus:     1  DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKKGQSPKLLIYFVSNRF  60
1A7:              1  ..........................................P....N.........  60

                     *                                  *********
VL consensus:    61  SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPWTFGGGTKLEIK          112
1A7:             61  ..................................................          112
```

```
                                                 *****            ***********
VH consensus:     1  QVQLKESGPGLVAPSQSLSITCTVSGFSLTSYGVHWVRQPPGKGLEWLGVIWGDGSTNYN  60
1A7:              1  ...V.....F..P.................T..S.I.............A.....T...H  60

                     *****                                ***********
VH consensus:    61  SALKSRLSISKDNSKSQVFLKMNSLQTDDTARYYCAExxxxYYAMDYWGQGTSVTVSS   119
1A7:             61  ...I.................L.........T....KL--GN.D.L.W...........  117
```

CURRICULUM VITAE

PERSONAL DATA

Name: Sunil K. Chatterjee, Ph.D.

Address: 2400 The Woods Lane
Lexington, KY-40502-6596

Date and Place of Birth: 8/7/40, Calcutta, India

Present Nationality: U.S. Citizen

Sex: Male

Marital Status: Married, two children

Wife's Name: Malaya Bhattacharya-Chatterjee, Ph.D.

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EDUCATION

Institute and Location	*Degree*	*Year*	*Major Field*
Presidency College, Calcutta, India	B.S.	1959	Chemistry
University of Calcutta, India	M.S.	1961	Biochemistry
University of Calcutta, India	Ph.D.	1966	Biochemistry

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Examiner: J. Reeves

Group Art Unit: 1813

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, MALAYA BHATTACHARYA-CHATTERJEE, Ph.D., do hereby declare as follows:

1. Under the name Malaya Chatterjee, I am an inventor for the above-referenced patent application.

2. I am a Member of the Markey Cancer Center in Lexington, and am an Associate Professor in the Department of Internal Medicine, University of Kentucky. My research expertise includes the fields of immunochemistry and molecular oncology. A copy of my *curriculum vitae*, describing my background and qualifications, accompanies this Declaration as *Exhibit A.*

3. In collaboration with the other inventors of the above-referenced patent application, I developed and cloned the 1A7 antibody-producing hybridoma cell line.

4. The cell line was obtained after repeated immunization of BALB/c mice with purified antibody 14G2a. Spleen cells from 4 immunized mice were fused with non-producing mouse myeloma cells and plated in 1200 wells. Supernatants from one of the wells was found to contain antibody reactivity specific for 14G2a but not for isotype or allotype controls. The antibody was also able to inhibit the binding of labeled 14G2a to GD2 expressed on a human cell line. The antibody-producing cells from this well were designated 1A7-1A1. The cells were subsequently cloned by two rounds of limiting dilution. This re-cloned line and the antibody produced thereby are referred to in the above-referenced patent application as 1A7.

5. Progeny of antibody-producing cells from the re-cloned line have been deposited with the ATCC under Accession No. BH-11786. Antibody from the cells has been characterized as described in the above-referenced patent application, and is claimed therein.

6. The 1A7-1A1 cells, the re-cloned 1A7 cell line, the predecessors and progeny thereof, and the antibody produced thereby has been maintained exclusively under the control of myself and the other inventors of the above-referenced application. The cells have been provided outside my laboratory to Dr. Sunil Chatterjee for purposes of ascertaining the sequence of the 1A7 variable region. The transfer was made with the agreement that the cells and the antibody not be redistributed, and that information on the sequence be kept confidential. Purified 1A7 antibody recently entered clinical trials at the University of Kentucky under strict supervision of

Dr. Ken Foon, co-inventor of the patent application. Neither the antibody nor the antibody-producing cells have been available to the public at any time.

7. The DNA and amino acid sequences of the 1A7 variable region genes were determined by Dr. Sunil Chatterjee under my auspices some time after the filing of the above-referenced patent application on January 7, 1995. The 1A7 sequence data have not been disclosed except under terms of confidentiality. The data were included in a recent grant application made to the National Institutes of Health under terms of confidentiality, and it is my understanding that the data will remain confidential until the grant is approved. It is my understanding that a decision on the application has not yet been rendered.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

3/11/96
Date

Malaya Bhattacharya-Chatterjee
Malaya Bhattacharya-Chatterjee, Ph.D.

CURRICULUM VITAE

I. General Information

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MARITAL STATUS: Married, two children

SPOUSE NAME: Sunil K. Chatterjee, Ph.D.

CHILDREN: Indranil Chatterjee (7/16/77)
Sumana Chatterjee (6/21/80)